Turk J Pharm Sci 2025;22(2):104-118
DOI: 10.4274/tjps.galenos.2025.12247

ORIGINAL ARTICLE

Comprehensive Investigation of Phytochemical Constituents and Biological Activities of *Scabiosa pseudograminifolia* Hub.-Mor.

Kübra ÖĞÜT[1]*, Gülmira ÖZEK[1], Nilgün ÖZTÜRK[1], Mehmet TEKİN[2], Temel ÖZEK[1],

[1]Anadolu University Faculty of Pharmacy, Department of Pharmacognosy, Eskişehir, Türkiye
[2]Trakya University Faculty of Pharmacy, Department of Pharmaceutical Botany, Edirne, Türkiye

ABSTRACT

Objectives: The aim of this study was to comprehensively investigate the phytochemical composition, including essential oils, fatty acids, and phenolic constituents, and to evaluate the antioxidant and α-amylase inhibitory activities of *Scabiosa pseudograminifolia* Hub.-Mor. (Caprifoliaceae), an endemic species growing in Sivas province of Türkiye. The plant materials were processed to obtain essential oils, and *n*-hexane, methanol, and aqueous extracts for chemical and biological evaluations.

Materials and Methods: Essential oils were obtained by hydrodistillation. Extracts were prepared using *n*-hexane, methanol, and water through maceration. The chemical compositions of the essential oil and fatty acids were analyzed using gas chromatography (GC)-mass spectrometry and GC-flame ionization detector (FID). Phenolic compounds were identified by reverse phase high performance liquid chromatography. Total phenolic and flavonoid contents, antioxidant activity [DPPH, Trolox Equivalent Antioxidant Capacity (TEAC), β-carotene bleaching, and Oxygen Radical Absorbance Capacity assays], and α-amylase inhibitory activity were all evaluated using spectrophotometric methods.

Results: Hexadecanoic acid (30.2%) and linalool (15.6%) were the main volatile compounds in the essential oil of *S. pseudograminifolia. (Z)*-3-Hexenal was the dominant leaf and flower volatile. The primary fatty acids were nonadecanoic and hexadecanoic acids. The aqueous extract exhibited the highest total phenolic (0.52±0.01 mg gallic acid equivalent/$g_{extract)}$ and flavonoid (0.081±0.002 mg quercetin equivalent/$g_{extract}$) contents. Among the tested samples, the essential oil showed the strongest TEAC value (2.39±0.15 mM), while the aqueous extract demonstrated potent antioxidant activity in DPPH (IC_{55}: 0.16±0.04 mg/mL) and β-carotene bleaching assays (inhibitory $concentration_{55}$: 0.730±0.001 mg/mL). The α-amylase inhibition levels of the extracts were found to be relatively low. Chlorogenic acid was the predominant phenolic compound.

Conclusion: This study presents the first phytochemical and biological investigation of *S. pseudograminifolia* Hub.-Mor., an endemic species from Türkiye. Essential oil analysis revealed hexadecanoic acid and linalool as major constituents, while nonadecanoic and hexadecanoic acids were predominant among the fatty acids. The methanol extract showed strong antioxidant activity, and chlorogenic acid was identified as a key phenolic compound. These findings support the potential of this species as a valuable source of natural antioxidants.

Keywords: *Scabiosa pseudograminifolia*, essential oil, fatty acids, phenolics, biological activity

INTRODUCTION

The genus *Scabiosa* L. is a member of the Dipsacaceae subfamily, which is a part of the *Caprifoliaceae* family.[1,2] Although it originated in the Mediterranean region and the Near East, it is a family that has spread to different regions, from Northern Europe to East Asia, from Central Africa to South Africa.[3] The genus *Scabiosa* encompasses a total of 80 species worldwide, including 43 found in Europe, while the remaining species are distributed across Africa and Asia.[4] About 34 *Scabiosa* species were recorded in the flora of Türkiye.[5] The nomenclature of the genus is derived from the Latin term "scabiosus or scabies," *Sarcoptes scabiei* L., known as the itching mite or scab beetle,

***Correspondence:** kubraogut@anadolu.edu.tr, **ORCID-ID:** orcid.org/0000-0002-9921-5866
Received: 25.09.2024 , **Accepted:** 16.02.2025 **Publication Date:** 14.05.2025

Cite this article as: ÖĞÜT K, ÖZEK G, ÖZTÜRK N, TEKİN M, ÖZEK T. Comprehensive investigation of phytochemical constituents and biological activities of *Scabiosa pseudograminifolia* Hub.-Mor. Turk J Pharm Sci. 2025;22(2):104-118

Copyright© 2025 The Author. Published by Galenos Publishing House on behalf of Turkish Pharmacists' Association.
This is an open access article under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 (CC BY-NC-ND) International License.

which causes highly contagious parasitic skin infections. It is thought to be given the name *Scabiosa* due to the use of multiple species for its treatment.[6] Although the members of the genus *Scabiosa* are generally known as "Uyuz Otu" in Türkiye, they have recently been called "Yazı Süpürgesi, Gicikotu, Kavurotu, Puk, Zivan".[7]

The literature review revealed that different species of the genus *Scabiosa* are commonly employed in traditional medicine to treat specific health issues. Specific species of *Scabiosa* are extensively utilized across various industries, including food, medicinal products, and skincare;[8] *S. columbaria* L. is traditionally employed for treating diphtheria, and *S. comosa* Fisch. Ex Roem. and Schult. is utilized in Mongolian and Tibetan traditional medicine for the treatment of liver ailments.[9] Also, qingganjiuwei powder, consisting of nine herbal components including *S. comosa*, is frequently used as an anti-fibrosis agent for patients with chronic liver disease in Inner Mongolia. This medication is approved by the Inner Mongolia Region Drug Administration for the treatment of liver disorders.[10] *S. atropurpurea* L. has been employed as a diuretic agent for acne, while S. succisa L. has been utilized in the treatment of asthma, bronchial pneumonia, and influenza. Furthermore, the external application of herbs from this particular species has been suggested for the treatment of respiratory, urogenital, and some skin conditions, such as herpes, ringworm, and scabies, as well as ulcers.[11] *S. stellata* L. is utilized for the treatment of heel fissures.[12] *S. tschilliensis* Grüning is utilized for hepatic disorders.[13]

With the phytochemical studies of a few *Scabiosa* species, the presence of coumarins, flavonoids, iridoids, pentacyclic triterpenoids, iridoid glucosides, and monoterpenoid glucoindole alkaloids has been reported.[9,14-19] Depending on this phytochemical content, the *Scabiosa* genus has demonstrated antidiabetic, hepatoprotective, analgesic, anti-inflammatory, antioxidant, antibacterial, anti-melanogenesis, anti-tyrosinase and anti-parasitic properties.[10]

To the best of our knowledge, no previous study has investigated the phytochemical composition or biological activities of *S. pseudograminifolia* Hub.-Mor., an endemic species growing in Türkiye. The aim of the study was to comprehensively evaluate the chemical composition, including essential oils, fatty acids, and phenolic constituents, and to assess the antioxidant and α-amylase inhibitory activities of different extracts of this species.

MATERIALS AND METHODS

Chemicals

The chemicals utilized in this study: *n*-hexane, dimethyl sulfoxide (DMSO), methanol, ethanol, formic acid, hydrochloric acid, glacial acetic acid, Folin-Ciocalteu (FC) reagent, boron trifluoride (BF_3) reagent, and butylated hydroxyanisole (BHA), were purchased from Sigma-Aldrich (USA). The lipid extraction kit, α-amylase produced from porcine pancreas (Type VI-B, ≥10 units/mg solid), and acarbose were purchased from Sigma (USA). The standard *n*-alkanes C_8-C_{40} were purchased from Fluka in Buchs. The phenolic acids utilized in this investigation, namely caffeic acid, chlorogenic acid, ferulic acid, gallic acid (GA), protocatechuic acid, p-hydroxybenzoic acid, syringic acid, and vanillic acid, along with propylparaben as an internal standard, were acquired from Sigma-Aldrich (St. Louis, MO, USA) or Merck (GmbH, Darmstadt, Germany). The rest of the chemicals utilized in this study were obtained from Merck (Germany).

Instruments

An Agilent 5975 gas chromatography-mass spectrometry (GC-MS) system manufactured by Agilent Technologies (Santa Clara, CA, USA) was used to conduct GC-MS analyses. The SPME technique for volatiles was performed using a manual SPME holder (57330-U, SUPELCO, Bellefonte, PA) and the polydimethylsiloxanedivinylbenzene 65 μm fiber (blue type). In the microtiter assays, the sample solutions were pipetted into microplate wells using an Eppendorf® Xplorer® 12-channel pipettor with a volume range of 10-300 μL. Two types of microplates were acquired from Sigma-Aldrich: a 96-well flat-bottom white polystyrene microplate, which was non-sterile (Greiner), and a 96-deep-well round-bottom polypropylene plate with a volume of 2.2 mL. The absorbance readings were recorded using a BioTek Powerwave XS microplate reader. Agilent 1100 series autosampler system from Agilent, GL Sciences Inc. (Waldbronn, Germany). The equipment was outfitted with a system controller, a DAD detector (G 1315B, 280 nm), and a quaternary LC pump (G1311A). The separation method was conducted using a Zorbax Eclipse XDB-C18 column (150 mm, 4.6 mm, 5 μm particle size), manufactured by Agilent in Waldbronn, Germany. The Human UP 9000 System (18 mW)'s water purification system provided ultrapure water. The liquid chromatographic system (Shimadzu LC 10Avp, Kyoto, Japan) had an in-line degasser, pump, and controller connected to an SPD-M10Avp photodiode array detector with an automatic injector and Class VP chromatography manager software. A reverse-phase C18 Ultrasphere column (INERTSIL, Waldbronn, Germany), (100x4.6 mm i.d. 3 microns) was used to analyze phenolic acids.

Plant material

The plant material *S. pseudograminifolia* Hub.-Mor. was collected at Sivas, Kangal-Gürün junction. The identification of the plant was conducted by Prof. Dr. Mehmet Tekin (Trakya University Faculty of Pharmacy, Department of Pharmaceutical Botany) and it was registered in Trakya University Faculty of Pharmacy Herbarium (code: 1621).

Hydrodistillation of essential oil

The essential oil was obtained from air-dried plant parts by hydrodistillation for 3 hours using clevenger-type equipment, following the techniques outlined in the European Pharmacopoeia.[20] The essential oil yield was calculated on a moisture-free basis. The oil underwent dehydration using anhydrous sodium sulfate and was thereafter preserved in amber glass vials at 4 °C until the gas chromatographic and biological activity analyses.

Microsteam distillation-solid phase microextraction (MSD-SPME)

In the experiment, 1.0 g of the leaf and flower parts were separately added to a 25 mL flask containing 3.0 mL of water.[21] A distillation head with a septum for SPME holder needle entrance and a condenser was attached to a flask that was designed explicitly for refluxing rather than distillation. In the pre-experiment, the fibre underwent conditioning at a temperature of 250 °C for 15 minutes. The electric heater was used gradually for the evaporation of volatiles from the sample. Once the evaporation started, the fibre was removed through the needle and placed in the headspace above the samples. The MSD-SPME process was conducted at the boiling point of water. The equilibrium time refers to the interval between the introduction of SPME fibre into the flask and the commencement of the extraction process. A sufficient extraction time of 3.0 minutes was employed following the establishment of equilibrium. Following the extraction time, the carefully loaded SPME fibre was withdrawn into the needle. Later, the needle was meticulously separated from the plug and employed for thermal desorption at the inlet port of gas chromatography-mass spectrometry (GC/MS) equipment.

Lipid extraction and fatty acid derivatization

Fatty acid research involved a series of consecutive steps, which included preparing the sample, extracting total lipids, methylating fatty acids, and then analyzing the fatty acid methyl esters using GC-MS/flame ionization detector (FID).[22] The lipid extraction kit was employed to extract the total lipids from the aerial parts of the plant material. The extraction of lipids requires a dual solvent partition mechanism, which comprises an aqueous and a lipophilic solvent (for example, chloroform). The lipids were retained in the lower layer of chloroform, whereas the water-soluble chemicals were retained in the upper layer of methanol-water. In the experiment, the mill-ground plant material (0.15 g) was homogenized in extraction solvent (3.0 mL) of the kit. Following the homogenization and vortexing, 0.5 mL of the buffer solution supplied in the kit was added to the mixture and vortexed. Following that, the organic solvent phase was filtered through a special filter of the kit. 200 µL of the extract was dried under nitrogen gas and then subjected to transesterification with BF_3-methanol reagent. The mixture was subjected to reflux for 1 h at 95 °C. After that, *n*-hexane (1.0 mL) and distilled water (1.0 mL) were added to the reaction vessel. The mixture was vortexed and centrifuged at 500 rpm for 5 minutes. The uppermost layer, hexane, was transported in a vial, concentrated under nitrogen gas, and thereafter injected into the GC-MS/FID system.

Preparation of extracts

The extracts of *S. pseudograminifolia* were prepared by fractionating the same powdered plant material with n-hexane, methanol, and water (plant material/solvent ratio 1:10). For each extract, the maceration process involved continuous shaking for 48x2 hours under ambient conditions. The supernatants obtained were filtered using Whatman filter paper. Subsequently, the organic solvents were removed from the filtrates using reduced pressure to obtain dry extracts. However, the aqueous extracts were dried using the lyophilization technique. The dried extracts were stored in amber glass vials at 4 °C until further analysis.[23,24]

The dried extracts were solved in 10% DMSO-methanol (10 mg/mL) and utilized as stock solutions before biological activity screening, total phenolic content assessment, and total flavonoid content evaluation.

GC/MS analysis

The GC-MS analysis was conducted using the previously stated settings.[25] An Agilent Innowax FSC column, 60 m x 0.25 mm, with a film thickness of 0.25 μm was used, along with a carrier flow rate of 0.8 mL/min. The GC oven was initially set at 60 °C for 10 minutes. It was then gradually increased to 220 °C at a rate of 4 °C per minute and held at that temperature for 10 minutes. Finally, the temperature was increased to 240 °C at a rate of 1 °C per minute. The split ratio was adjusted to 40:1, while the injector temperature was set to 250 °C. The MS spectra were recorded at 70 eV, covering a mass range of 35 to 450 *m/z*.

Gas chromatography analysis

The GC assay was conducted with Agilent 6890N GC equipment. To achieve an equivalent elution sequence as observed in GC-MS, the elution line was divided between MS and FID detectors, and a single injection was conducted using the same column and suitable operational parameters. The temperature of the FID was set at 300 °C. The contents of the essential oil and fatty acid methyl esters were determined by co-injecting them with standards procured commercially or obtained from pure organic sources whenever feasible. Furthermore, the confirmation of compound identities was achieved through the comparison of their mass spectra with records available in the Wiley-NIST GC/MS Library (Wiley, NY, USA), MassFinder software 4.0 (Dr. Hochmuth Scientific Consulting, Hamburg, Germany, and Adams Library".[26] Confirmation was accomplished by utilizing the exclusive Başer Library of Essential Oil Constituents database, which was acquired through chromatographic investigations conducted on pure compounds under identical equipment and conditions. For confirmation of identified compounds, each of the compounds' relative retention indices was calculated with a C8-C40 *n*-alkane standard mixture (Fluka, Buchs, Switzerland). The FID chromatograms were used to calculate the relative percentage of the separated individual compounds without normalization.

Reverse phase high performance liquid chromatography (RP-HPLC) analysis

The extracts of *S. pseudograminifolia were subjected to RP-HPLC analysis to get* a profile of the phenolic acids. The chromatographic separation was conducted using two solvent systems: (A) a mixture of methanol, water, and formic acid in a ratio of 10:88:2 *(v/v/v)*, (B) a mixture of methanol, water, and formic acid in a ratio of 90:8:2 *(v/v/v)*, as reported previously.[27] The study employed a gradient elution program in the following manner: from 15-20 min, to 85% A; from 20-30 min, to 50% A;

from 30-35 min, to 0% A; and from 36-42 min, back to 100% A. The flow rate was 1 mL/min, and the injection volume was 10 µL. Signals were detected at 280 nm. The relevant extracts were dissolved in a mixture of methanol and water (1:1, v/v) and injected into the HPLC.

The peaks were identified using the following method: the separate phenolic acid standards were dissolved. The rate of peak normalization (peak area/peak retention time) of the relevant phenolic acids, was determined by calculating the integrated peak areas and their corresponding retention times. The quantities of these phenolic acids were then measured in the associated extracts using their calibration curves.

Total phenol content

The extracts of *S. pseudograminifolia* were evaluated for the total phenolic content measured as gallic acid (GA) equivalent (GAE) using a Folin-Ciocalteu (FC) reagent, according to previous procedure.[28] Methanol was used to prepare the stock solutions of the extracts and GA. The experimental procedure involved the combination of 20 µL of the sample solution (extract/GA), 1560 µL of ultrapure water, and 100 µL of FC reagent into a 96 deep-well plate. Following an incubation period of 1-8 minutes, 300 µL of a sodium carbonate solution (20%) was added to the mixture. The mixture was subjected to a 2-hour incubation period (at 25 °C in the dark). Subsequently, 300 µL of the mixture was put into a 96-well microplate. The absorbance readings at a wavelength of 760 nm were subsequently compared to a GA calibration curve, which was established using a 5-point calibration range spanning from 0.01 to 1.0 mg/mL. The experiment was repeated three times. The calibration curve for GA had a regression coefficient r^2 of 0.9992, calculated as y=0.7489x+0.0551. The results were expressed as mg GAE/$g_{extract,}$ and values are presented as mean ± standard deviation (SD) from triplicate experiments.

Total flavonoid content

The extracts of *S. pseudograminifolia* were evaluated for the total flavonoid content measured as quercetin equivalent (QE) using aluminum chloride as a reagent. In the experiment, 80 µL of the sample solution (extract/quercetin), 80 µL of $AlCl_3$, and 1840 µL of absolute ethanol were added into 96-deep-well. In the blank samples, 10 µL of acetic acid (15%) was added instead of aluminum chloride. Following an incubation period of 40 minutes, 300 µL of the mixture was transferred into the 96-well microplate. The absorbance values were measured at a wavelength of 415 nm using a microplate reader. The quantification of total flavonoid content was performed using a calibration curve based on quercetin. A 5-point calibration was used to plot the calibration curve within the concentration range of 0.01-1.0 mg/mL.[29] The calibration curve for quercetin had a regression coefficient r^2 of 0.9996, calculated as y = 1.857x + 0.0088. The average content value was computed using a standard error of ±. The results were quantitatively represented as mg QE/$g_{extract}$.

Trolox Equivalent Antioxidant Activity (TEAC) Test

The antioxidant potential of the extracts and essential oils of *S. pseudograminifolia* was assessed using ABTS+•, respectively, and calculated as Trolox equivalent.[30] At first, a solution of ABTS+• (7 mM) and potassium persulfate was prepared in pure water. Following a 16-hour incubation period in the dark, the aliquot of the solution was diluted with absolute ethanol until it reached an absorbance range of 0.700-0.800 at a wavelength of 734 nm. The extracts, essential oil (2 mg/mL), and Trolox (five dilutions ranging from 3.0 to 0.125 mM) were made in methanol (with 10% DMSO) as stock solutions. The experiment involved combining 10 µL sample (essential oil, extract, Trolox) with a 990 µL ABTS solution in a 96-deep well plate. Following a 30-minute incubation in the dark, a decrease in absorbance was recorded at a wavelength of 734 nm using a microplate reader. The ABTS•+ scavenging activity of the samples was quantified as TEAC and was determined using a linear equation calculated for Trolox (y=29.997x-0.6918). The calibration curve's regression coefficient was calculated to be r^2=0.9989. The experiment was conducted three times.

Free Radical Scavenging Effect (DPPH) Test

The samples' ability to scavenge DPPH radicals was assessed using a modified version of the Brand-Williams method.[31] The extracts, essential oil (2 mg/mL), and standard inhibitor (0.1 mg/mL) were prepared in methanol with 10% DMSO as stock solutions. In the experiment, 100 µL of the sample solution was combined with 100 µL of DPPH solution (0.08 mg/mL in MeOH) in 96-well flat-bottom plate cells. The mixtures were incubated in the dark for 30 minutes. The reduction in absorbance at a wavelength of 517 nm was recorded with the microplate reader. The gallic acid solution was employed in this test as the positive control. The experiments were conducted three times. The samples' free radical scavenging activity was quantified as a percentage of inhibition, which was determined using the following equation:

$$\%\,Inh = \left(\frac{Abs_{control} - Abs_{sample}}{Abs_{control}}\right) \times 100$$

The absorbance of the control includes all reagents except the test substance, is indicated as $Abs_{control.}$ Abs_{sample} represents the absorbance of the sample after the addition of DPPH (Version 12.0).

Lipid Peroxidation Inhibition (β-Carotene Bleaching Test)

The present study aimed to assess the inhibitory impact of extracts and essential oils on lipid peroxidation using the β-carotene bleaching test with some modifications.[32] A chloroform solution containing 1 mg/mL of β-carotene was prepared and subsequently combined with 200 mg of Tween-20 and 25 µL of linoleic acid to generate an emulsion. Chloroform was completely evaporated using a vacuum (at a temperature of 40 °C) and then nitrogen gas. Following that, oxygenated ultrapure water (50 mL) was added, and the mixture was vortexed.

Before each experiment, the emulsion solution was freshly prepared and stored in the dark. The standard antioxidant employed in the study was BHA. The stock solutions of the

samples were prepared in methanol (containing 10% DMSO) at a concentration of 5 mg/mL. In the experiment, 60 µL of a sample (extract/essential oil/BHA) was combined with 250 µL of an emulsion solution within a 96-well plate. The absorbance values were measured at 50 °C every 15 minutes for a total of 105 minutes at a wavelength of 492 nm. The experiments were conducted three times. The results of the experiment were calculated using the following formula:

$$\%\,\mathrm{AA} = \left(1 - \frac{(Abs_{0\,sample} - Abs_{120\,sample})}{(Abs_{0\,control} - Abs_{120\,control})}\right) \times 100$$

Where AA is the antioxidant activity, $Abs_{0\ sample}$ and Abs_{120} sample are the absorbance values of the sample at 0 min and 120 min, and Abs_0 control and Abs_{120} control are the absorbance values of the control at 0 min and 120 min. In this context, AA represents the antioxidant activity, whereas $Abs_{0\ sample}$ and $Abs_{120\ sample}$ indicate the absorbance values of the sample at 0 minutes and 120 minutes, respectively. Similarly, $Abs_{0\ control}$ and $Abs_{120\ control}$ represent the absorbance values of the control at 0 minutes and 120 minutes.

Oxygen Radical Absorbance Capacity (ORAC) Test

The oxygen radical absorption capacity of the extracts and essential oil of *S. pseudograminifolia* was assessed using a microtiter assay[33] with certain modifications. A fluorescent probe was employed in the assay, while 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) was utilised as a free radical generator. The standard antioxidant employed in the study was Trolox. The stock solution was prepared by dissolving 15 mg of fluorescein in 10 mL of phosphate buffer solution (0.075 M, pH 7.4). The resulting solution was stored in the dark at 4 °C. In a 96-well plate, 25 µL of a sample (extract/essential oil/trolox) and 150 µL of fluorescein solution were combined and incubated for 30 minutes at 37 °C in the dark. The plate was shaken for 10 seconds following the addition of 25 µL of AAPH reagent after incubation. The fluorescence values were measured at 37 °C at 60-second intervals over 180 minutes. The excitation wavelength was 485 nm, and the emission wavelength was 535 nm. The under the curve (AUC) values were determined using the *SigmaPlot* program. The net AUC values were obtained using the following formula:

NET AUC= AUC_{sample}-AUC_{blank}

The standard curve was derived by graphing the net AUC and the linear relationship between the concentration of Trolox and net AUC. The calculation of ORAC values was performed using Trolox equivalents. The experiments were conducted three times.

α-Amylase Inhibition Test

Following the previously reported assay,[34] of the extracts and essential oil were tested for their capacity to inhibit the α-amylase enzyme. The inhibitor of the α-amylase enzyme employed in this study was acarbose. The extract and essential oil solutions were prepared in methanol (containing 10% DMSO) in appropriate quantities. For the experiment, 50 µL of the sample (extract/essential oil/acarbose) and 50 µL of an enzyme solution (0.8 U/mL in 20 mM sodium phosphate buffer pH 6.9) were added to a 96-well plate. The mixture was then incubated for 10 minutes in the dark at 37 °C. Following the incubation period, 50 µL of a starch solution (0.05%) was added, and the resulting mixture was incubated for an additional 10 minutes in the dark at 37 °C. Following the incubation period, the reaction was terminated by adding 25 µL of HCl solution (1M). Finally, 100 µL of I_2/KI reagent solution was added to the wells. In the absence of an enzyme solution, a buffer solution was added to the blank samples. All the reagents were present in the control wells except for the sample. The plate reader was used to record absorbance readings at a wavelength of 630 nm. The calculation of the inhibition was done using the following formula:

$$\mathrm{Inh} = \left(\frac{(Abs_{0\,control} - Abs_{control\,blank}) - (Abs_{sample} - Abs_{sample\,blank})}{(Abs_{0\,control} - Abs_{control\,blank})}\right) \times 100$$

Statistical analysis

Quantitative data obtained from the biological activity assays were statistically evaluated. All antioxidant and α-amylase inhibition experiments were performed in triplicate, and the results are presented as mean ± standard deviation. Statistical differences between the samples were assessed using one-way ANOVA followed by Tukey's multiple comparison test, with significance accepted at $p<0.05$. Analyses were carried out using IBM SPSS Statistics version 21, developed by IBM Corp., headquartered in New York, USA.

RESULTS

The phytochemical profile of *S. pseudograminifolia* and its *in vitro* antioxidant and anti-α-amylase effects have been determined for the first time within the scope of this investigation. All these studies have contributed to closing the knowledge gap regarding *S. pseudograminifolia*. The leaves of *S. pseudograminifolia* have undergone extraction using solvents of different polarities, namely *n*-hexane, methanol, and aqueous solution. Table 1 displays the extract yields.

Chemical composition of essential oil and volatile constituents

The constituents that make up the essential oil from *S. pseudograminifolia* aerial parts have been identified using GC-MS and GC-FID techniques, and the results are presented in Table 2 and Supplementary Figure 1. In addition to this, the

Table 1. Yields of extracts

Extract type	Extract code	Amounts of extract, g	Yield*, %
n-Hexane extract	SP_H	0.151	0.43
Methanol extract	SP_M	4.733	13.44
Aqueous extract	SP_W	3.057	8.68
Essential oil	SP_{EO}	0.008	0.02

*Yield was calculated based on air dried plant weight

Table 2. Hydrodistilled essential oil compounds and volatile constituents obtained from *S. pseudograminifolia* aerial parts, leaves, and flowers using the MSD-SPME technique

RRIexp.	RRIlit.	Compound	%			References
			Hydrodistilled aerial parts	MSD-SPME of leaves	MSD-SPME of flowers	
945	945	2-Ethyl furan	0.3			35
950	949	2,4-Dimethylfuran	0.7			36
1047	1050	(*E*)-2-Butenal		0.8	0.5	37
1052	-	4-Methyl 2,4-pentadienal#	0.8			
1087	1098	Hexanal		0.5	4.4	38
1187	1187	Heptanal			0.6	39
1191	1149	(*E*)-3-Hexenal	1.2			39
1202	1198	Limonene		0.7	1.2	40
1211	1215	Isoamyl alcohol			1.5	41
1212	1211	1,8-Cineole		0.1		40
1223	1244	Amyl furan		0.1		42
1225	1225	(*Z*)-3-Hexenal		34.0	29.1	40
1230	1231	(*E*)-2-Hexenal			1.5	43
1251	1253	γ-Terpinene			0.1	44
1256	1231	Isocumene	8.0			45
1260	1262	(E)-β-Ocimene		0.2	0.2	44
1263	1260	Pentanol			0.2	46
1285	1280	*p*-Cymene		0.2	0.3	47
1295	1282	Terpinolene		0.2	0.3	40
1306	1287	Octanal		0.2	0.3	40
1319	1319	4-Nonanone			0.2	48
1331	1332	(*E*)-2-Heptenal			0.3	37
1332	1356	Allyl caproate		0.6		49
1344	1337	6-Methyl-5-hepten-2-one		1.3	0.6	40
1363	1351	Hexanol		1.7	7.7	40
1377	1340	(*E*)-3-Hexene-1-ol			0.2	50
1400	1400	Tetradecane		0.4		42
1403	1380	(*Z*)-3-Hexene-1-ol		0.9	2.0	40
1407	1391	2-Nonanone			0.1	40
1409	1392	Nonanal		1.1	0.6	40
1410	1400	(*E*)-2-Hexene-1-ol			0.7	40
1412	-	3-Octene-2-one#			0.4	51
1413	1388	4,8-Dimethyl-1,3,7-nonatriene		1.3		42
1443	1428	(*E*)-2-Octenal		0.4	0.4	52

combined MSD-SPME technique was utilized to investigate the plant's volatile constituents. The plant's leaves and flowers were analyzed separately for this purpose. The hydrodistilled essential oil compounds, in the order in which they were eluted

Table 2. Continued

RRIexp.	RRIlit.	Compound	% Hydrodistilled aerial parts	MSD-SPME of leaves	MSD-SPME of flowers	References
1450	1444	1-Octen-3-ol		0.1	0.4	[40]
1454	1446	*trans*-Linalool oxide		0.2	0.3	[40]
1463	1449	Heptanol			0.2	[40]
1471	1479	*(E,Z)*-2,4-Heptadienal		1.9	0.4	[42]
1476	1461	Furfural			0.3	[40]
1496	1490	2-Ethyl hexanol		3.2	4.3	[53]
1499	1496	Decanal			0.3	[40]
1502	1497	*(E,E)*-2,4-Heptadienal		1.5	0.8	[53]
1514	1535	β-Burbonene		0.4		[54]
1520	1524	*(E,Z)*-3,5-Octadiene-2-one		0.9	0.8	[55]
1528	1515	Camphor		0.3	0.2	[40]
1541	1519	Benzaldehyde		1.8	3.9	[40]
1553	1543	Linalool	15.6	3.1	5.0	[40]
1559	1552	Octanol		1.3	1.1	[40]
1568	1573	*(E,E)*-3,5-Octadiene-2-one		0.5	0.6	[56]
1586	1566	(*E*,*E*)-2,6-Nonadienal			0.6	[47]
1589	1583	(*E*,*Z*)-2,6-Nonadienal			0.3	[57]
1600	1600	Hexadecane		0.5		[58]
1612	1614	Furfuryl alcohol		1.1		[59]
1619	-	4-Methyl-(2E)-undecene#		3.6	2.5	[39]
1630	-	6-Methyl-1-octanol#		4.3	2.5	[39]
1632	1638	β-Cyclocitral		1.7		[60]
1641	1616	1-Ethyl-1H-pyrrole-2-carbaldehyde#			0.2	[61]
1660	1656	Nonanol		2.9	2.1	[40]
1662	1665	Safranal		0.8		[62]
1663	1663	Phenylacetaldehyde		0.5	2.1	[63]
1678	1648	Acetophenone			0.7	[40]
1702	1670	Methyl chavicol		0.7	0.2	[40]
1719	1706	α-Terpineol	2.5	0.7	0.9	[64]
1726	1712	Dodecanal			0.5	[40]
1743	-	2-(1-Cyclopenten-1-yl) furan#		0.7		[39]
1748	-	Methoxy phenyl oxime#		1.0	0.8	[39]
1770	1751	Carvone		0.2	0.4	[65]
1775	1753	Ethyl benzaldehyde		0.3	0.3	[66]
1790	1762	Naphthalene		0.6	0.4	[67]

Table 2. Continued

RRIexp.	RRIlit.	Compound	%			References
			Hydrodistilled aerial parts	MSD-SPME of leaves	MSD-SPME of flowers	
1796	-	Octenyl cyclopentene#		2.8		39
1797	2025	*(E)*-Cinnamaldehyde			0.2	68
1807	1797	Methyl acetophenone§		0.4	0.4	69
1815	1775	Cuminaldehyde		1.2	0.9	70
1816	1839	Geraniol	2.3			40
1824	-	2,2,4-Trimethyl-3-carboxyisopropyl-isobutyl pentanoate#	0.9			39
1842	1845	*(E)*-Anethole		2.5	2.0	60
1860	1867	*(E)*-Geranyl acetone		1.2	0.3	71
1872	-	2,2,4-Trimethyl pentane-1,3-diol diisobutyrate#			0.2	39
1876	-	2-Naphthalenol		0.3		39
1878	1880	1-Isobutyl 4-isopropyl 3-isopropyl-2,2-dimethylsuccinate		0.6		58
1886	1872	2-Methyl naphthalene		0.1		72
1894	1865	Benzyl alcohol		0.4	0.2	40
1941	1904	Phenylethyl alcohol			0.4	40
1964	1936	(E)-β-Ionone		1.6	0.2	40
1995	1996	Benzothiazole		0.3	0.2	73
2022	1992	Phenol			0.1	40
2023	1995	trans-β-Ionone-5,6-epoxide		0.5		42
2025	2028	Methyl eugenol		0.2	0.1	74
2036	2041	Isopropyl myristate		0.1		75
2037	2015	Pentyl octyl benzene		0.1		76
2041	2038	Phenyl ether		0.2		77
2065	2058	Anisaldehyde		0.2	0.2	78
2066	2072	Lilial		0.2	0.1	79
2130	2131	Hexahydro farnesyl acetone	3.0	0.4	0.4	80
2144	2120	*(Z)*-3-Hexen-1-ol benzoate		0.1		81
2182	2179	3,4-Dimethyl-5-pentylidene-2(5H)-furanone		0.6	0.2	42
2194	2192	Nonanoic acid *(=Pelargonic acid)*	1.5			82
2202	2105	Thymol		0.2	0.2	83
2243	2240	Carvacrol	1.3	3.0	1.9	83
2451	2449	Dodecanoic acid *(=Lauric acid)*	10.9			84

Table 2. Continued

RRIexp.	RRIlit.	Compound	% Hydrodistilled aerial parts	MSD-SPME of leaves	MSD-SPME of flowers	References
2670	2670	Tetradecanoic acid *(=Myristic acid)*	9.1			[85]
2820	2822	Pentadecanoic acid *(=Pentadecylic acid)*	1.6			[86]
2877	2890	Hexadecanoic acid (=*Palmitic acid)*	30.2			[87]
		Total	89.9	96.7	94.7	

[#]: Tentative identification from Wiley-NIST digital library, MSD-SPME: Microsteam distillation-solid phase microextraction, RRI_{exp}: Experimentally calculated retention index, RRI_{lit}: Retention index from literature

on the HP-Innowax FSC column, along with their relative percentages, retention indices, and method of identification, are presented in Supplementary Figures 2 and 3, and in Table 2, as well as volatile constituents extracted with the MSD-SPME technique.

Fatty acid chemical composition

This research enabled the initial assessment of the fatty acid composition of *S. pseudograminifolia*. The lipids of *S. pseudograminifolia* were isolated from the leaves and flowers with microscale techniques and subjected to transesterification with BF_3 reagent for subsequent GS-FID/MS analysis. Results of chromatographic separation and identification of the methyl ester derivatives are presented in Supplementary Figures 4 and 5, and Table 3, respectively.

Total phenolic, flavonoid contents and biological activity results

The phenolic compounds make an important contribution to the total antioxidant potential of natural products. So, basically, their phenolic contents were specified, and the characterization of the extracts was to be determined. The total phenolic contents of *S. pseudograminifolia* extracts were assessed using a FC reagent, and the results were presented in terms of GA equivalent. The total flavonoid content of *S. pseudograminifolia* extracts was assessed as the QEs. The results of spectrophotometric assays are presented in Table 4.

HPLC analysis results

Some phenolic acids in the *S. pseudograminifolia* extracts have been determined using an RP-HPLC gradient system with a modified technique.[27] The HPLC results of *S. pseudograminifolia* aqueous and methanol extracts are shown in Table 5. The amounts are given in $\mu g/g_{extract}$.

DISCUSSION

Chemical composition of essential oil and volatile constituents

In the GC-MS study of *S. pseudograminifolia,* seventeen compounds accounted for 89.9% of the total identified components. Hexadecanoic acid (30.2%), linalool (15.6%), and dodecanoic acid (10.9%) are the main components of the essential oil from the of *S. pseudograminifolia* aerial parts derived through the hydrodistillation.

We also used the MSD-SPME technique to examine volatile components of leaves and flowers. From the leaves of *S. pseudograminifolia*, we found 68 volatile constituents, which made up 96.7% of all the components found using the MSD-SPME. The main volatile components were *(Z)*-3-hexenal (34.0%), 6-methyl-1-octanol (4.3%), 4-methyl-(2*E*) undecene (3.6%), and linalool (3.1%). Additionally, from the flowers of *S. pseudograminifolia*, we found 72 volatile constituents, which made up 95% of all the components found using the MSD-SPME. The main volatile components were *(Z)*-3-hexenal (29.1%), hexanol (7.7%), linalool (5%), and hexanal (4.4%). Table 2 presents these findings.

In the published literature, there are few gas chromatographic investigations of essential oils from aerial parts *Scabiosa* species. In *S. columbaria* subsp. *columbaria* var. *columbaria* L. the major volatile compounds, were identified as 4-octadecenal (30.0%) in the flower and carvone (35.44%) in the leaf.[88] The main components of the essential oil were tricosane (15.5%), rosifoliol (15.3%), (*E*)-caryophyllene (10.7%), and α-humulene (7.9%) in the aerial parts of *S. flavida* Boiss. & Hausskn.[89] In the leaf essential oil of *S. maritima* L., the main components were hexahydrofarnesyl acetone (42.0%) and dodecanoic acid (17.2%). In the inflorescence of *S. maritima,* the main components of essential oil were 3-vinyl pyridine (23.5%) and hexahydrofarnesyl acetone (19.4%).[90] α-Thujone (34.4%), camphor (17.5%), and β-thujone (15.29%) constituted the major compounds of the fruit oil of *S. arenaria* Forssk from Tunisia, while chrysanthenone (23.4%), together with camphor (12.9%) and α-thujone (10.7%), were the main constituents essential oil of the leaf and stem. In the case of the flower oil, also chrysanthenone (38.5%), camphor (11.7%), and α-thujone (9.5%) were reported as the major compounds.[4]

The volatile components of the aerial parts was compared to that of leaves and flowers, revealing that they did not have the same major compounds. In addition, it has been observed that the chemical composition of *S. pseudoraminifolia* differs from that of other *Scabiosa* species.

Fatty acid chemical composition

A total of 20 fatty acids, accounting for 99.8% of the total oil content, were identified in the leaves of *S. pseudograminifolia*, while 23 fatty acids were detected in the flower samples, representing 78.3% of the total oil. The predominant components in the leaves were methyl nonadecanoate (21.9%), methyl hexadecanoate (20.8%), and *(Z,Z,Z)*-9,12,15-methyl octadecatrienoate. In the flowers, methyl hexadecanoate

(18.3%), *(Z,Z)*-9,12-methyl octadecadienoate (12.4%), and *(Z,Z,Z)*-9,12,15-methyl octadecatrienoate (12.2%) were the major constituents.

According to the literature, the leaves of *Scabiosa* species typically contain up to 19 fatty acids, ranging from lauric acid (C12:0) to nervonic acid (C24:1n9). Palmitic acid (C16:0), behenic acid (C22:0), lignoceric acid (C24:0), and linoleic acid (C18:3n6) are commonly reported as the dominant fatty acids. The reported proportion of saturated fatty acids ranges from 48.97% to 80.11%, while unsaturated fatty acids range from 13.62% to 25.39% (Table 3).

The detection of 20 and 23 fatty acids in the leaves and flowers of *S. pseudograminifolia*, respectively, indicates a slightly broader fatty acid diversity compared to other species in the genus. Notably, the high proportion of methyl nonadecanoate (C19:0) observed in the leaf extract-a component rarely emphasized in previous *Scabiosa* studies-may serve as a chemotaxonomic marker specific to this species. Additionally, the predominance of saturated fatty acids in both organs aligns with the characteristic lipid composition previously documented for the genus.

Total phenolic, flavonoid contents and biological activity results

An assessment was conducted to determine the antioxidant activity of the essential oil of *S. pseudograminifolia* and its *n*-hexane, methanolic, and aqueous extracts. The test results included the DPPH free radical scavenging effect, TEAC, ORAC, and β-carotene peroxidation inhibition assay (Table 4).

There is a scarcity of reports regarding phenolic and flavonoid contents of *Scabiosa* species. The overall phenolic content of *S. arenaria* varied from 34.77 to 269.09 mg $GAE/g_{extract}$, while

Table 3. Chemical composition of the fatty acids obtained from the leaves and flowers of the *S. pseudograminifolia*

RRI#	Compound	Leaves	Flowers
1402	Methyl octanoate (=*Caprylic acid methyl ester*); (8:0)		0.3
1505	Methyl nonanoate (=*Pelargonic acid methyl ester*); (9:0)		0.3
1815	Methyl dodecanoate (=*Lauric acid methyl ester*); (12:0)	0.9	0.5
1980	*Unidentified* (MA:278)	0.4	0.2
2012	*Unidentified* (MA:278)	1.0	0.5
2016	Methyl tetradecanoate (=*Myristic acid methyl ester*); (14:0)	1.9	1.0
2051	Octanedioic acid (=*Suberic acid*); (8:0)		0.4
2125	Methyl pentadecanoate (=*Pentadecylic acid methyl ester*); (15:0)	0.5	0.5
2158	Nonanedioic acid (=*Azelaic acid*); (9:0)	1.0	1.0
2223	Methyl hexadecanoate (=*Palmitic acid methyl ester*); (16:0)	20.8	18.3
2251	*(Z)*-9-Methyl hexadecenoate (=*Palmitoleic acid methyl ester*); (16:1); ω-7		0.4
2330	Methyl heptadecanoate (=*Margaric acid methyl ester*); (17:0)	0.6	0.4
2436	Methyl octadecanoate (=*Stearic acid methyl ester*); (18:0)	7.1	5.6
2455	*(Z)*-9-Methyl octadecenoate (=*Oleic acid methyl ester*); (18:1); ω-9	7.8	4.0
2468	*(E)*-9-Methyl octadecenoate (=*Elaidic acid methyl ester*); (18:1); ω-9	0.6	0.9
2509	*(Z,Z)*-9,12-Methyl octadecadienoate (=*Linoleic acid methyl ester*); (18:2); ω-6	5.8	12.4
2542	Methyl nonadecanoate (=*Nonadecylic acid methyl ester*); (19:0)	21.9	
2572	*(Z,Z,Z)*-9,12,15-Methyl octadecatrienoate (=*α-Linolenic acid methyl ester*); *(18:3)*; ω-3	10.3	12.2
2642	Methyl eicosanoate (=*Arachidic acid methyl ester*); (20:0)	4.4	3.1
2740	Methyl heneicosanoate (=*Heneicosilicic acid methyl ester*); (21:0)	0.6	0.6
2843	Methyl docosanoate (=*Behanic acid methyl ester*); (22:0)	8.3	6.5
2868	*(Z)*-13-Methyl dococenoate (=*Erucic acid methyl ester*); (22:1); ω-9	1.2	5.0
2945	Methyl trichosanoate (=*Trichosilic acid methyl ester*); (23:0)	0.8	0.5
3050	Methyl tetracosanoate (=*Lignoceric acid methyl ester*); (24:0)	3.9	3.7
	Total	99.8	78.3

RRI# : Relative retention index of the methyl/ethyl derivative of the compound

Table 4. TPC, TFC and biological activity results of *S. pseudograminifolia* extracts

Codes	TPC (mgGAE/g $_{extract}$)	TFC (mgQE/g $_{extract}$)	DPPH (IC_{50}, mg/mL)	TEAC (mM)	ORAC[a)] (TEμmol)	β-Carotene peroxidation inhibition (IC_{50}, mg/mL)	α-Amylase inhibition (%)
SP_{EO}	-	-	NE	2.39±0.15	32.6±6.8	NE	33.6
SP_{H}	0.11±0.06	NE	28.80±1.90[b)]	0.26±0.14	NE	NE	NE
SP_{M}	0.50±0.01	0.067±0.008	0.19±0.03	2.21±0.20	134.0±11.0	0.730±0.001	NE
SP_{W}	0.52±0.01	0.081±0.002	0.16±0.04	2.33±0.13	248.4±15.4	1.4±0.2	NE
BHA	-	-	-	-	-	0.01±0.0005	-
GA	-	-	0.002±0.00	-	-	-	-
ACR	-	-	-	-	-	-	85.0

[a)]: ORAC values are determined for essential oil and extracts at 0.1 mg/mL, [b)]: IC_{50} was not calculated; the value was for 10 mg/mL, ORAC: Oxygen Radical Absorbance Capacity, TPC: Total phenol content, TFC: Total flavonoid content, SP_{EO}: The plant's essential oil, SP_{H}: The plant's *n*-hexane extract, SP_{M}: The plant's methanol extract, SP_{W}: The plant's aqueous extract code, BHA: Butylated hydroxyanisole, GA: Gallic acid, ACR: Acarbose, NE: Non-effective, TEAC: Trolox equivalent antioxidant activity.

Table 5. RP-HPLC quantitative determination of phenolic acids in *S. pseudograminifolia* extracts

	Phenolic acid amounts (μg/$g_{extract}$)							
Extracts	PA	*p*-HBA	CA	ClA	SA	*p*-CA	FA	*o*-CA
SP_{M}	0.11	0.12	0.05	4.06	0.07	0.44	0.30	0.53
SP_{W}	0.22	0.79	0.25	5.26	0.21	0.89	0.48	0.65

RP-HPLC: Reverse phase high performance liquid chromatography, SP_{M}: The plant's methanol extract, SP_{W}: The plant's aqueous extract code, PA: Protocatechic acid, *p*-HBA: *p*-Hydroxybenzoic acid, CA: Caffeic acid, ClA: Chlorogenic acid, SA: Syringic acid, *p*-CA: *p*-Coumaric acid, FA: Ferulic acid, *o*-CA: *o*-Coumaric acid

the quantities of flavonoid compounds ranged from 0.81 to 10.9 mg QE/$g_{extract}$. Previously, it has been reported that the aqueous methanol extract and fractions from *S. atropurpurea* subsp. *maritima* contained the total phenolic content ranged from 17.7 to 186.75 mg GAE/$g_{extract}$, and the total flavonoid content varied from 4.38 to 208.69 mg catechin equivalent/g. The methanolic extract of *S. sicula* was found to have a total phenolic content of 2.67 mg GAE/$g_{extract}$. In the methanolic extracts of *S. columbaria* subsp. *columbaria* var. *columbaria* from Türkiye, the phenolic content ranged from 269.833 to 640.111 μg GAE/mL, while the flavonoid content ranged from 6.060 to 13.527 μg QAE/mL.[14] In our study, we detected that the aqueous extract of *S. pseudograminifolia* exhibited the highest concentrations of phenols and flavonoids: 0.52±0.01 mg GAE/$g_{extract}$ and 0.081±0.002 mg QE/$g_{extract}$, respectively.

The literature demonstrated a strong suppression of DPPH free radicals in the aqueous extract of *S. arenaria*, with an inhibitory concentration $(IC)_{50}$ value of 0.18 mg/mL.[92] The crude extracts of *S. tschiliensis* exhibited DPPH-scavenging action, with an IC_{50} value of 25.68±1.21 μg/mL. They had much more DPPH-scavenging power than other plants from the *Scabiosa* genus that were studied, like *S. comosa* and *S. arenaria*.[93] According to the literature, the IC_{50} values for the crude extract of *S. atropurpurea* ranged from 22.42 to 415.23 mg/mL. The crude extract obtained from the leaves exhibited the most significant DPPH-scavenging activity compared to other plant parts.[94] In this study, we found that the extract's IC_{50} values ranged from 0.16 to 0.19 mg/mL, indicating no activity of the essential oil towards DPPH free radicals. The TEAC value of the methanol extract from *S. sicula* was found to be 0.34±0.01 μg/mL. This is the concentration of Trolox solution that has the same antioxidant activity as a 1 mg/mL solution of the extract.[95] The TEAC value of *S. arenaria* was 0.56 mM Trolox/$g_{extract}$,[92] leaf parts of *S. columbaria*, it was 267.381±0.012 and 242.857±0.003, respectively.[88] The essential oil (2.39±0.15) and, aqueous extract (2.33±0.13) had the highest TEAC values, respectively, in contrast to DPPH activity. ORAC tests in *Scabiosa* species are rare and are the first in the literature to be performed in hexane, methanol, and water extracts, with the highest activity observed in water extract. In terms of β-carotene bleaching kinetics, it was observed that the methanolic fraction of *S. atropurpurea* and ascorbic acid (the reference compound) exhibited the highest level of efficacy in inhibiting β-carotene oxidation. These curves were nearly identical and showed their respective inhibition percentages were strikingly similar, with values of 97.19% and 100%. The findings indicate that the *n*-hexane and chloroform extracts exhibit a moderate level of antioxidant activity, with individual levels of 64.17% and 42.91%, respectively.[96] The inhibition values for the methanol extract were 0.730±0.001 mg/mL, and those for the water extract were 1.4±0.2 mg/mL. The study revealed a significant positive connection between the antioxidant activity and the total phenolic content of each extract. The α-amylase enzyme-inhibiting effect of *S. pseudograminifolia* was found to be insufficient

HPLC analysis results

The analysis revealed that *S. pseudograminifolia* has significant amounts of chlorogenic acid, caffeic acid, protocatechic acid, *p*-coumaric acid, syringic acid, and ferulic acid (Table 5). Regarding the biological activity of extracts, the study revealed a significant positive connection between the activity and the total phenolic content of each extract. Therefore, in this investigation, we have looked at the phenolic compounds in *S. pseudograminifolia*'s methanolic and aqueous extracts as a whole.

There are quite enough studies on the phenolic composition of *Scabiosa* species in the literature. These investigations have revealed that isoorientin and 4-O-caffeoylquinic acid are the primary compounds found in *S. stellata* extract.[97] Additionally, caffeoylquinic acid, rutin, ursolic acid, cyanuric acid, sinapic acid, luteolin, apigenin, quercetin, kaempherol, and tamarixetin have also been identified. Gallic, chlorogenic, caffeic, syringic, *p*-coumaric, sinapic, ferulic, catechin hydrate, epicatechin-3-*O*-gallate, luteolin-7-*O*-glucoside, isorhamnetin 3-*O*-glucoside, rutin, isoquercetin, myricetin, kaempferol-3-*O*-rutinoside, isorhamnetin-3-*O*-rutinoside, quercetin, naringenin, luteolin, isorhamnetin, and apigenin were detected in *S. atropurpurea* subsp. *maritima* hydromethanol extract.[94]

The methanol extracts from *S. columbaria* subsp. *columbaria* var. *columbaria* contained six phenolic compounds that were identified as the main ingredients. These were GA, catechin, 4-OH-benzoic acid, 4-OH-benzaldehyde, caffeic acid, and chlorogenic acid. The methanolic extract derived from *S. columbaria* subsp. *columbaria* contains GA and caffeic acid.[88]

CONCLUSION

This research includes the first biological activity and phytochemical studies on *S. pseudograminifolia* Hub.-Mor. growing in Sivas province of Türkiye. The main constituents of the essential oil derived from *S. pseudograminifolia* were hexadecanoic acid (30.2%), linalool (15.6%), and dodecanoic acid (10.9%). The MSD-SPME method revealed that *(Z)*-3-hexenal (34.0%), 2-ethyl hexanol (3.2%), 6-methyl-1-octanol (4.3%), 4-methyl-(2*E*) undecene (3.6%), and linalool (3.1%) were were the major volatile components in the leaves of *S. pseudograminifolia*. Similarly, *(Z)*-3-hexenal (29.1%), hexanol (7.7%), linalool (5%), and hexanal (4.4%) were identified as the main volatile components in the flowers. Non-adecanoic (21.9%) and hexadecanoic (20.8%) acids were found as fatty acids in *S. pseudograminifolia*. The aqueous and methanol extracts exhibited a significant concentration of chlorogenic acid. The highest TEAC values were determined for essential oil and aqueous extract. The methanol extract of *S. pseudograminifolia* exhibited the highest levels of β-carotene peroxidation inhibition, and the aqueous extract exhibited the highest levels free radical scavenging potential. The plant's ability to inhibit α-amylase enzyme is insufficient. The elucidation of the phytochemical content and bioactivity potential of *S. pseudograminifolia* extracts supports their potential use in further pharmacological studies and contributes to the growing knowledge on underexplored endemic flora.

Ethics

Ethics Committee Approval: Not required.

Informed Consent: Not required.

Footnotes

Authorship Contributions

Concept: K.Ö., T.Ö., Design: K.Ö., T.Ö. Data Collection or Processing: K.Ö., G.Ö., N.Ö., T.Ö., M.T., Analysis or Interpretation: K.Ö., G.Ö., N.Ö., T.Ö., Literature Search: K.Ö., Writing: K.Ö., G.Ö., N.Ö., T.Ö.

Conflict of Interest: The authors declare no conflicts of interest.

Financial Disclosure: The research was financially supported by the Scientific Research Projects Department of Anadolu University (BAP No: 2103S009).

REFERENCES

1. Akimaliev A. β-sitosterol and oleanolic acid from Scabiosa soongorica. Chem Nat Compd. 1977;13:596.
2. Akyol Y, Kocabaş O, Kayacan E, Minareci E, Özdemir C. Scabiosa hispidula Boiss. (Caprifoliaceae) türüne ait anatomik bir çalışma. Nevşehir Bilim Teknol Derg. 2016;5:10-15.
3. Heywood VH. Flowering plants of the world. Oxford; Oxford University Press; 1978:239-240.
4. Besbes M, Omri A, Cheraif I, Daami M, Ben Jannet H, Mastouri M, Aouni M, Selmi B. Chemical composition and antimicrobial activity of essential oils from Scabiosa arenaria Forssk. growing wild in Tunisia. Chem Biodivers. 2012;9:829-839.
5. TÜBİVES. [cited 2021 Dec 21]. Available from: http://194.27.225.161/yasin/tubives/index.php?sayfa=hizli_ara
6. Aksoy A, Çelik J, Bozkurt M, Uysal T. Türkiye florası için yeni bir tür kaydı: Scabiosa lucida Vill. (Caprifoliaceae). Bağbahçe Bilim Derg. 2020;7:49-57.
7. Menemen Y. Türkçe Bilimsel Bitki Adları Yönergesi'nde değişiklik ve ilave öneriler. Bağbahçe Bilim Derg. 2019;6:1-3.
8. Besbes Hlila M, Omri A, Ben Jannet H, Lamari A, Aouni M, Selmi B. Phenolic composition, antioxidant and anti-acetylcholinesterase activities of the Tunisian Scabiosa arenaria. Pharm Biol. 2013;51:525-532.
9. Pinto DCGA, Rahmouni N, Beghidja N, Silva AMS. Scabiosa genus: a rich source of bioactive metabolites. Medicines (Basel). 2018;5:110.
10. Skała E, Szopa A. Dipsacus and Scabiosa species-the source of specialized metabolites with high biological relevance: A review. Molecules. 2023;28:3754.
11. Besbes Hlila M, Mosbah H, Majouli K, Ben Nejma A, Ben Jannet H, Mastouri M, Mahjoub Aouni, Boulbaba Selmi. Antimicrobial activity of Scabiosa arenaria Forssk. extracts and pure compounds using bioguided fractionation. Chem Biodivers. 2016;13:1262-1272.
12. Bammi J, Douira A. Les plantes médicinales dans la forêt de l'achach (plateau central, Maroc). 2002.
13. Chen Q, Wang Y, Ma F, Han M, Wang Z, Xue P, Lu J. Systematic profiling of the effective ingredients and mechanism of Scabiosa comosa and S. tschilliensis against hepatic fibrosis combined with network pharmacology. Sci Rep. 2021;11:2600.
14. Albayrak S, Aksoy A, Yilmaz MA, Beyzi E. Investigation of phytochemical, antioxidant and antidiabetic potentials of Scabiosa L.

(Caprifoliaceae) species with chemometric methods. Chem Biodivers. 2024;21:202301652.

15. Perdetzoglou D, Skaltsa H, Tzakou O, Harvala C. Comparative phytochemical and morphological study of two species of the Scabiosa L. genus. Feddes Repert. 1994;105:157-165.
16. Tundis R, Loizzo MR, Menichini F, Statti GA, Menichini F. Biological and pharmacological activities of iridoids: recent developments. Mini Rev Med Chem. 2008;8:399-420.
17. Ghisalberti EL. Biological and pharmacological activity of naturally occurring iridoids and secoiridoids. Phytomedicine. 1998;5:147-163.
18. Kılınc H, Masullo M, Lauro G, D'Urso G, Alankus O, Bifulco G, Piacente S. Scabiosa atropurpurea: A rich source of iridoids with α-glucosidase inhibitory activity evaluated by in vitro and in silico studies. Phytochemistry. 2023;205:113471.
19. Otang-Mbeng W, Sagbo IJ. Anti-melanogenesis, antioxidant and anti-tyrosinase activities of Scabiosa columbaria L. Processes. 2020;8:236.
20. European Pharmacopoeia. Essential Oils in Herbal Drugs, in: European Pharmacopoeia, 11th edition, p. 323-324, European Directorate for the Quality of Medicines & HealthCare (EDQM), Council of Europe, Strasbourg, France, 2023. 2023:323-324.
21. Göger G, Türkyolu Ü, Gürşen EN, Yur S, Karaduman AB, Göger F, Özek G. Phytochemical characterisation of Phlomis linearis Boiss. & Bal and screening foranticholinesterase, antiamylase, antimicrobial, and cytotoxic properties. Turk J Chem. 2021;45:387-399.
22. Möller R, Nürnberg G, Albrecht E, Ruth W, Brockmann GA, Dannenberger D. A method for analyzing fatty acids in cattle hair, with special emphasis on lauric acid and myristic acid. Eur J Lipid Sci Technol. 2019;121:1900143.
23. Göğer F, Özek G, Tekin M, Yur S, Özek T. phytochemical profiling and evaluation of Marrubium sivasense Aytaç, Akgül & Ekici for antioxidant activity and ınhibition effects on α-amylase, lipoxygenase, xanthine oxidase and tyrosinase enzymes. JOTCSA. 2019;6:281-292.
24. Özek G, Özbek M, Yur S, Göger F, Arslan M, Özek T. Assessment of endemic Cota fulvida (Asteraceae) for phytochemical composition and ınhibitory activities against oxidation, α-amylase, lipoxygenase, xanthine oxidase and tyrosinase enzymes. Records of Nat Prod. 2019;13:333-345.
25. Özek G, Özek T, Başer KHC, Duran A, Sagiroglu M, Duman H. Comparison of the essential oils of Prangos turcica A. Duran, M. Sagiroglu et H. Duman fruits obtained by different isolation techniques. J Essent Oil Res. 2006;18:511-514.
26. Adams RP. Identification of essential oil components by gas chromatography/mass spectrometry. Carol Stream 2007;456:544-545.
27. Öztürk N, Tuncel M, Tuncel NB. Determination of phenolic acids by a modified HPLC: Its application to various plant materials. J Liq Chromatogr Relat Technol. 2007;30:587-596.
28. Vl S. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 1999;299:152-178.
29. Yur S, Tekin M, Göger F, Başer KHC, Özek T, Özek G. Composition and potential of Tanacetum haussknechtii Bornm. Grierson as antioxidant and inhibitor of acetylcholinesterase, tyrosinase, and α-amylase enzymes. Int J Food Prop. 2017;20:2359-2378.
30. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med. 1999;26:1231-1237.
31. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. Food Sci Technol. 1999;28:1231-1237.
32. Baderschneider B, Luthria D, Waterhouse AL, Winterhalter P. Antioxidants in white wine (cv. Riesling): I. Comparison of different testing methods for antioxidant activity. Vitis. 1999;38:127-131.
33. Gillespie KM, Chae JM, Ainsworth EA. Rapid measurement of total antioxidant capacity in plants. Nat Protoc. 2007;2:867-870.
34. Zengin G, Sarikurkcu C, Aktumsek A, Ceylan R. Sideritis galatica Bornm.: a source of multifunctional agents for the management of oxidative damage, Alzheimer's and diabetes mellitus. J Funct Foods. 2014;11:538-547.
35. Brunton NP, Cronin DA, Monahan FJ. Volatile components associated with freshly cooked and oxidized off-flavours in turkey breast meat. Flavour Fragr J. 2002;17:327-334.
36. Narain N, de Sousa Galvao M, Madruga MS. Volatile compounds captured through purge and trap technique in caja-umbu (Spondias sp.) fruits during maturation. Food Chem. 2007;102:726-731.
37. Hashizume M, Gordon MH, Mottram DS. Light-induced off-flavor development in cloudy apple juice. J Agric Food Chem. 2007;55:9177-9182.
38. Héberger K, Görgényi M. Principal component analysis of Kováts indices for carbonyl compounds in capillary gas chromatography. J Chromatogr A. 1999;845:21-31.
39. NIST Mass Spectrometry Data Center. NIST Mass Spectrometry Data Center, William E. Wallace, director, "Retention Indices" in NIST Chemistry WebBook, NIST Standard Reference Database Number 69, Eds. P.J. Linstrom and W.G. Mallard, National Institute of Standards and Technology, Gaithersburg MD, 20899, https://doi.org/10.18434/T4D303, (retrieved August 26, 2024). 2024.
40. Babushok VI, Linstrom PJ, Zenkevich IG. Retention indices for frequently reported compounds of plant essential oils. J Phys Chem Ref Data. 2011;40.
41. Kang HR, Hwang HJ, Lee JE, Kim HR. Quantitative analysis of volatile flavor components in Korean alcoholic beverage and Japanese sake using SPME-GC/MS. Food Sci Biotechnol. 2016;25:979-985.
42. Shukurlu EN, Özek G, Özek T, Vitalini S. Chemical composition of different plant parts from Lactuca serriola L.-focus on volatile compounds and fatty acid profile. Z Naturforsch C J Biosci. 2023;78:285-291.
43. Koprivnjak O, Conte L, Totis N. Influence of olive fruit storage in bags on oil quality and composition of volatile compounds. Food Technol Biotechnol. 2002;40:129-134.
44. Frizzo CD, Serafini LA, Dellacassa E, Lorenzo D, Moyna P. Essential oil of Baccharis uncinella DC. from Southern Brazil. Flavour Fragr J. 2001;16:286-268.
45. Censullo AC, Jones DR, Wills MT. Speciation of the volatile organic compounds (VOCs) in solventborne aerosol coatings by solid phase microextraction-gas chromatography. J Coat Technol. 2003;75:47-53.
46. Feng Z, Wu X, Chen V, Velie E, Zhang Z. Incidence and survival of desmoplastic melanoma in the United States, 1992-2007. J Cutan Pathol. 2011;38:616-624.
47. Menichini F, Tundis R, Bonesi M, De Cindio B, Loizzo MR, Conforti F, GA Statti., Menabeni R, Bettini R, and Menichini F. Chemical composition and bioactivity of Citrus medica L. cv. Diamante essential oil obtained by hydrodistillation, cold-pressing and supercritical carbon dioxide extraction. Nat Prod Res. 2011;25:789-799.
48. Saini R, Jaitak V, Guleria S, Kaul VK, Kiran Babu GD, Singh B, Lal B, Singh RD. Comparison of headspace analysis of volatile constituents

with GCMS analysis of hydrodistilled and supercritical fluid extracted oil of Capillipedium parviflorum. J Essent Oil Res. 2012;24:315-320.

49. Vinogradov BA. Production, composition, properties and application of essential oils, 2004, retrieved from http://viness.narod.ru. 2004.
50. Huang J, Yang M, Zong Y, Liu C, Yu H, Chen C, Tian H. Characterization of the aroma in barley leaves from different cultivars and tillering stages by HS-SPME/GC-MS, GC-O and E-nose. Food Biosci. 2024;60:104117.
51. Hu S, Ren H, Song Y, Liu F, Meng L. Analysis of volatile compounds by GCMS reveals the geographical origin of cultivated rice. 2022.
52. Thakeow P, Angeli S, Weißbecker B, Schütz S. Antennal and behavioral responses of Cis boleti to fungal odor of Trametes gibbosa. Chem Senses. 2008;33:379-387.
53. Feng T, Zhuang H, Ye R, Jin Z, Xu X, Xie Z. Analysis of volatile compounds of Mesona blumes gum/rice extrudates via GC-MS and electronic nose. Sens Actuators B Chem. 2011;160:964-973.
54. Rochat S, Egger J, Chaintreau A. Strategy for the identification of key odorants: application to shrimp aroma. J Chromatogr A. 2009;1216:6424-6432.
55. Yamaguchi K, Shibamoto T. Volatile constituents of green tea, Gyokuro (Camellia sinensis L. var Yabukita). J Agric Food Chem. 1981;29:366-370.
56. Altintas A, Kose YB, Yucel E, Demirci B, Baser KHC. Composition of the essential oil of Centaurea dichroa. Chem Nat Compd. 2004;40:604-605.
57. Senger-Emonnot P, Rochard S, Pellegrin F, George G, Fernandez X, Lizzani-Cuvelier L. Odour active aroma compounds of sea fig (Microcosmus sulcatus). Food Chem. 2006;97:465-471.
58. Tekin M, Özek G, Martin E, Özek T, Yılmaz G, Başer KHC. Essential oil characterization of Cousinia sivasica Hub.-Mor. Asteraceae Biol Divers Conserv. 2018;11:16-21.
59. Shibamoto T, Kamiya Y, Mihara S. Isolation and identification of volatile compounds in cooked meat: sukiyaki. J Agric Food Chem. 1981;29:57-63.
60. Kaya A, Başer KHC, Koca F. Essential oils of Acinos troodi (Post) Leblebici subsp. vardaranus Leblebici and subsp. grandiflorus Hartvig & Strid. Flavour Fragr J. 1999;14:50-54.
61. Welke JE, Manfroi V, Zanus M, Lazarotto M, Zini CA. Characterization of the volatile profile of Brazilian Merlot wines through comprehensive two-dimensional gas chromatography time-of-flight mass spectrometric detection. J Chromatogr A. 2012;1226:124-139.
62. Liao X, Yan J, Wang B, Meng Q, Zhang L, Tong H. Identification of key odorants responsible for cooked corn-like aroma of green teas made by tea cultivar "Zhonghuang 1". Food Res Int. 2020;136:109355.
63. Özek G, Chidibayeva A, Ametov A, Nurmahanova A, Özek T. Chemical composition of flower volatiles and seeds fatty acids of Rosa iliensis Chrshan, an endemic species from Kazakhstan. Records of Natural Products. 2022;16:225-235.
64. Schepetkin IA, Özek G, Özek T, Kirpotina LN, Kokorina PI, Khlebnikov AI, Quinn MT. Neutrophil immunomodulatory activity of nerolidol, a major component of essential oils from Populus balsamifera buds and propolis. Plants. 2022;11:3399.
65. Nyiligira E, Viljoen AM, Başer KHC, Özek T, Van Vuuren SF, Houghton PJ. Essential oil composition and in vitro antimicrobial and anti-inflammatory activity of South African Vitex species. South African Journal of Botany. 2004;70:611-617.
66. Le Guen S, Prost C, Demaimay M. Characterization of odorant compounds of mussels (Mytilus edulis) according to their origin using gas chromatography–olfactometry and gas chromatography–mass spectrometry. J Chromatogr A. 2000;896:361-371.
67. Wong KC, Tie DY. The essential oil of the leaves of Murraya koenigii Spreng. Journal of Essential Oil Research. 1993;5:371-374.
68. Sandner D, Krings U, Berger RG. Volatiles from Cinnamomum cassia buds. Z Naturforsch C J Biosci. 2018;73:67-75.
69. Schepetkin IA, Özek G, Özek T, Kirpotina LN, Khlebnikov AI, Quinn MT. Neutrophil immunomodulatory activity of (−)-borneol, a major component of essential oils extracted from Grindelia squarrosa. Molecules. 2022;27:4897.
70. Hachicha SF, Skanji T, Barrek S, Ghrabi ZG, Zarrouk H. Composition of the essential oil of Teucrium ramosissimum Desf. (Lamiaceae) from Tunisia. Flavour Fragr J. 2007;22:101-104.
71. Kundakovic T, Fokialakis N, Kovacevic N, Chinou I. Essential oil composition of Achillea lingulata and A. umbellata. Flavour Fragr J. 2007;22:184-187.
72. Soria AC, Sanz J, Martínez-Castro I. SPME followed by GC-MS: a powerful technique for qualitative analysis of honey volatiles. European Food Research and Technology. 2009;228:579-590.
73. Başer KHC, Özek G, Özek T, Duran A. Composition of the essential oil of Centaurea huber-morathii Wagenitz isolated from seeds by microdistillation. Flavour Fragr J. 2006;21:568-570.
74. Christensen LP, Jakobsen HB, Paulsen E, Hodal L, Andersen KE. Airborne Compositae dermatitis: monoterpenes and no parthenolide are released from flowering Tanacetum parthenium (feverfew) plants. Arch Dermatol Res. 1999;291:425-431.
75. Pozo-Bayón MA, Ruíz-Rodríguez A, Pernin K, Cayot N. Influence of eggs on the aroma composition of a sponge cake and on the aroma release in model studies on flavored sponge cakes. J Agric Food Chem. 2007;55:1418-1426.
76. Peng CT, Hua RL, Maltby D. Prediction of retention indexes: IV. Chain branching in alkylbenzene isomers with C10-13 alkyl chains identified in a scintillator solvent. J Chromatogr. 1992;589:231-239.
77. Johanningsmeier SD, McFeeters RF. Detection of volatile spoilage metabolites in fermented cucumbers using nontargeted, comprehensive 2-dimensional gas chromatography-time-of-flight mass spectrometry (GC×GC-TOFMS). J Food Sci. 2011;76:168-177.
78. Xiao Z, Dai S, Niu Y, Yu H, Zhu J, Tian H, Gu Y. Discrimination of Chinese vinegars based on headspace solid-phase microextraction-gas chromatography mass spectrometry of volatile compounds and multivariate analysis. J Food Sci. 2011;76:1125-1135.
79. Polatoğlu K, Arsal S, Demirci B, Başer KHC. Unexpected Irregular Monoterpene "Yomogi Alcohol" in the Volatiles of the Lathyrus L. species (Leguminosae) of Cyprus. J Oleo Sci. 2016;65:241-249.
80. Formisano C, Rigano D, Napolitano F, Senatore F, Arnold NA, Piozzi F, Rosselli S. Volatile constituents of Calamintha origanifolia Boiss. growing wild in Lebanon. Nat Prod Commun. 2007;2:1253-1256.
81. Bisio A, Ciarallo G, Romussi G, Fontana N, Mascolo N, Capasso R, Biscardi D. Chemical composition of essential oils from some Salvia species. Phytotherapy Research. An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. 1998;12:117-120.

82. Suleimenov YM, Atazhanova GA, Ozek T, Demirci B, Kulyjasov AT, Adekenov SM, Baser KHC. Essential oil composition of three species of Achillea from Kazakhstan. Chem Nat Compd. 2001;37:447-450.
83. Başer KHC, Özek T, Demirci B, Kürkçüoğlu M, Aytaç Z, Duman H. Composition of the essential oils of Zosima absinthifolia (Vent.) Link and Ferula elaeochytris Korovin from Turkey. Flavour Fragr J. 2000;15:371-372.
84. Selli S, Cabaroglu T, Canbas A, Erten H, Nurgel C, Lepoutre JP, Gunata Z. Volatile composition of red wine from cv. Kalecik Karası grown in central Anatolia. Food Chem. 2004;85:207-213.
85. Başer KHC, Özek G, Özek T, Duran A, Duman H. Composition of the essential oils of Rhabdosciadium oligocarpum (Post ex Boiss.) Hedge et Lamond and Rhabdosciadium microcalycinum Hand-Mazz. Flavour Fragr J. 2006;21:650-655.
86. Kaya A, Başer KHC, Tümen G, Koca F. The essential oil of Acinos suaveolens (Sm.) G. Don fil. Acinos arvensis (Lam.) Dandy and Acinos rotundifolius Pers. growing wild in Turkey. Flavour Fragr J. 1999;14:60-64.
87. Quijano CE, Linares D, Pino JA. Changes in volatile compounds of fermented cereza agria [Phyllanthus acidus (L.) Skeels] fruit. Flavour Fragr J. 2007;22:392-394.
88. Akar Z. Chemical compositions by using LC-MS/MS and GC-MS and antioxidant activities of methanolic extracts from leaf and flower parts of Scabiosa columbaria subsp. columbaria var. Columbaria L. Saudi J Biol Sci. 2021;28:6639-6644.
89. Javidnia K, Miri R, Javidnia A. Constituents of the essential oil of Scabiosa flavida from Iran. Chem Nat Compd. 2006;42:529-530.
90. Jalloul A Ben, Garzoli S, Chaar H, el Jribi C, Abderrabba M. Seasonal effect on bioactive compounds recovery using aqueous extraction, antioxidant activities, and volatile profiles of different parts of Scabiosa maritima L.(=Scabiosa atropurpurea subsp. maritima (L.) Arcang.). South African Journal of Botany. 2023;152:63-79.
91. Ebadi-Nahari M, Farnia P, Nikzat S, Mollaei S. A chemotaxonomic evaluation of some Scabiosa L. species in Iran. Biochem Syst Ecol. 2018;81:33-36.
92. Hafsa M Ben, Besbes M, Mahjoub MA, Mighri Z. Antimicrobial, antioxidant activities and total phenolic content of the acetonic, ethanolic and aqueous extracts of Scabiosa arenaria (Forsk). J Med Plants Nat Prod. 2009;1:59-68.
93. Wang J, Liu K, Li X, Bi K, Zhang Y, Huang J, Zhang R. Variation of active constituents and antioxidant activity in Scabiosa tschiliensis Grunning from different stages. J Food Sci Technol. 2017;54:2288-2295.
94. Ben Jalloul A, Chaar H, Tounsi MS, Abderrabba M. Variations in phenolic composition and antioxidant activities of Scabiosa maritima (Scabiosa atropurpurea sub. maritima L.) crude extracts and fractions according to growth stage and plant part. South African Journal of Botany. 2022;146:703-714.
95. Kılınç H, Masullo M, D'Urso G, Karayildirim T, Alankus O, Piacente S. Phytochemical investigation of Scabiosa sicula guided by a preliminary HPLC-ESI-MSn profiling. Phytochemistry. 2020;174:112350.
96. Ali-Rachedi F, Laoud A, Meraghni M. Antioxidant activity and acetylcholinesterase inhibitory of Scabiosa atraupurpuria sub maritima L. Extract. Research Journal of Biotechnology. 2024;19:57-63.
97. Rahmouni N, Pinto DCGA, Beghidja N, Benayache S, Silva AMS. Scabiosa stellata L. phenolic content clarifies its antioxidant activity. Molecules. 2018;23:1285.

Click the link to access Supplementary Figure 1-5: https://d2v96fxpocvxx.cloudfront.net/68f48462-babf-415b-b878-1e392d1c83af/content-images/f74cb961-a41f-4861-97c7-10bdf2156076.pdf